Supercritical Fluid Extraction

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Supercritical Fluids

It is now 170 years since Baron Charles Cagniard de la Tour discovered that, above a certain temperature, single substances do not condense or evaporate, but exist only as fluids. In the following decades the 'critical point' was characterized, with its parameters: the critical temperature and pressure. In recent years fluids have been widely exploited at conditions above, but not too far removed from, their critical temperatures and pressures. The term 'supercritical fluids' has been coined to describe these media. Their value lies in the fact that they can have properties intermediate between those we associate with gases and liquids, and also that the properties can be controlled by pressure as well as temperature. Consequently, supercritical fluids can often provide optimum conditions for both experiments and processes. Equally important, especially as regulations become tougher, is that supercritical fluids offer environmental advantages. This is mainly because carbon dioxide and water are available as solvents. The disadvantages of supercritical fluids are that high pressures and sometimes temperatures are involved, and, in the case of water, there are corrosion problems. As the technology to overcome them is available, these disadvantages become cost and convenience factors to weigh against potential advantages. Consequently, supercritical fluids are being exploited in specialized areas. Amongst these is supercritical fluid extraction (SFE), on both an industrial and analytical scale.

Substances used as supercritical fluids include hydrocarbons, such as propane and ethene, water and ammonia, fluorinated hydrocarbons and even xenon. However, one compound, carbon dioxide, has so far been the most widely used in extraction, because of its convenient critical temperature, cheapness, chemical stability, non-flammability, stability in radioactive applications and non-toxicity. Large amounts of carbon dioxide released accidentally could constitute a working hazard, given its tendency to blanket the ground, but hazard detectors are available. It is an environmentally friendly substitute for other organic solvents. The carbon dioxide that is used is obtained in large quantities as a by-product of fermentation, combustion and ammonia synthesis and would be released into the atmosphere sooner rather than later, if it were not used as a supercritical fluid. Its polar character as a solvent is intermediate between a truly non-polar solvent such as hexane and weakly polar solvents. Because the molecule is non-polar it is often classified as a non-polar solvent, but it has some limited affinity with polar solutes because of its large molecular quadrupole. It has a particular affinity for fluorinated compounds and is useful for working with fluorinated metal complexes and fluoropolymers.

To increase the affinity of carbon dioxide to a variety of solutes, substances are added as 'modifiers' or 'entrainers'. The characteristics they impart include increased or decreased polarity, aromaticity, chirality, and the ability to further complex metal-organic compounds. For example, methanol is added to increase polarity, aliphatic hydrocarbons to decrease it, toluene to impart aromaticity, [R]-2-butanol to add chirality, and tributyl phosphate to enhance the solvation of metal complexes. They are often added in 5% or 10% amounts by volume, but sometimes much more, say 50%. They can have significant effects when added in small quantities and in these cases it may be the effect on surface processes rather than solvent character which is important. For example, the modifier may be effective in extraction by adsorbing onto surface sites, preventing the readsorption of a compound being extracted.

Because supercritical fluid have properties intermediate between those of gases and liquids to an extent controlled by pressure, optimum conditions can be sought for extraction. The medium can be adjusted for compounds to be sufficiently soluble to be removed, while at the same time the viscosity and diffusion coefficients can be high enough to bring about relatively rapid mass transport. **Table 1** shows typical values for the density and viscosity of a gas, supercritical fluid and liquid, taking carbon dioxide as an example. Density is more than half that of the liquid, giving rise to reasonable solubility. Moreover, by controlling the solvent density SFE can, to some extent, be made selective. In contrast, however, the viscosity of a supercritical fluid is much closer to that of a gas than that of a liquid. Thus pressure drop through a supercritical extraction cell is less than for the equivalent liquid process. Diffusion coefficients, also shown in Table 1 for naphthalene in carbon dioxide, are higher in a supercritical fluid than in a liquid. They are approximately inversely related to the fluid density. The advantage shown in the table is seen not to be so great and the main diffusional advantage lies in the fact that typical supercritical solvents have smaller molecules than typical liquid solvents. The diffusion coefficient for naphthalene in

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Table 1 The density, ρ, and viscosity, η, of carbon dioxide and the diffusion coefficient for naphthalene in carbon dioxide, D, under gas, supercritical and liquid conditions

	ρ/kg m^{-3}	η/μPa s	D/m^2 s^{-1}
Gas, 313 K, 1 bar	2	16	5.1×10^{-6}
Supercritical, 313 K, 100 bar	632	17	1.4×10^{-8}
Liquid, 300 K, 500 bar	1029	133	8.7×10^{-9}

a typical liquid would be closer to $1 \times 10^{-9}\,m^2\,s^{-1}$. Thus diffusion coefficients in supercritical fluid experiments and processes are typically an order of magnitude higher than in a liquid medium. This has the advantage of faster transport in the narrow passages typical in an extraction.

Application to Extraction

Because of the properties of a supercritical fluid, as described above, SFE can be more rapid than liquid extraction. Furthermore, the solvent is removed more easily, and fractionation of the extract by reducing the pressure in stages is feasible. SFE was first exploited on a process scale and this application continues to develop. On an industrial scale the first and most famous example is the 'natural' decaffeination of green coffee beans by the Hag process initiated in Bremen. Hops are also extracted by SFE on a large scale. Apart from these large-scale processes, more than 30 high-value oils, flavours and essences are extracted commercially in batch processes.

SFE is also used in chemical analysis to replace liquid extraction for sample preparation for a wide range of systems. SFE is now being used for the Total Diet Study programme of the US Food and Drug Administration. Usually, SFE is more rapid, less laborious and involves solvents which are less hazardous. Efforts still have to be made to make it more quantitative, but in fairness to SFE, extraction is often incomplete using a liquid. SFE is sometimes used online with an analytical method such as gas chromatography; it is most successful for some polymer and plant extractions. **Table 2** summarizes the principle analytical applications of SFE.

Laboratory-Scale SFE

SFE is carried out on a laboratory scale for both sample preparation and for initial studies on possible industrial processes. A range of commercial equipment is available to carry out experimental studies conveniently. A simple system is shown schematically in **Figure 1**. It can be assembled in-house and shows the principles involved. The fluid, typically carbon dioxide, is supplied from a cylinder with a dip tube to a pump, which can be a pump designed for liquid chromatography capable of delivering up to 5 mL per minute at a pressure of 400 bar and displaying the pressure and the flow rate. The pump head must be accessible so that it can be cooled by circulating an ethylene glycol and water mixture from a cooler, so that the fluid substance is pumped as a liquid. An alternative method of ensuring this is to use a fluid supply with an overhead pressure of around 100 bar of helium. In this case, cooling the pump head is not necessary, but the fluid will contain a small percentage of helium. The pumped fluid substance then passes into a controlled heater, which can be an oven for gas chromatography. It first passes through a length (typically 0.3 m) of stainless steel tubing into an extraction cell, rated for 400 bar at 100°C, which is fitted with a frit at the exit end (often both ends) to keep the sample matrix to be extracted in place. The exit tube is then connected to a restrictor to maintain the pressure in the system. This can be of stainless steel or, alternatively, a quartz capillary, in which case the connector will have a graphitized ferrule. The effluent then passes through a collecting solvent to trap the extracted compounds. Because of the cooling effect as the fluid expands to atmospheric pressure, it is usually necessary to heat the restrictor and the simplest way of doing this is with a domestic hair dryer. Evaporation of the collecting solvent may occur and it will be necessary to add solvent to the vial during the extraction. This simple device, although often satisfactory, can suffer from blocking of the

Table 2 Examples of the use of SFE in analytical sample preparation

Matrix	*Examples of analytes extracted*
Soils, sludges, water	Agrochemicals, polychlorobiphenyls, polycyclic aromatic hydrocarbons, fuel hydrocarbons, phenols, surfactants, metals
Food and animal tissue	Veterinary drugs, pesticides, anabolic steroids, mycotoxins, fats
Human milk and serum	Drugs
Polymers, food packaging	Low oligomers, polymer additives
Herbs, cosmetic products	Flavours, fragrances
Plant tissue	Alkaloids, various natural products, triglycerides
Fly ash, engine emissions	Polycyclic aromatic hydrocarbons, dioxins
Sedimentary rocks	Biomarker hydrocarbons
Fermentation broths	Biologically active compounds

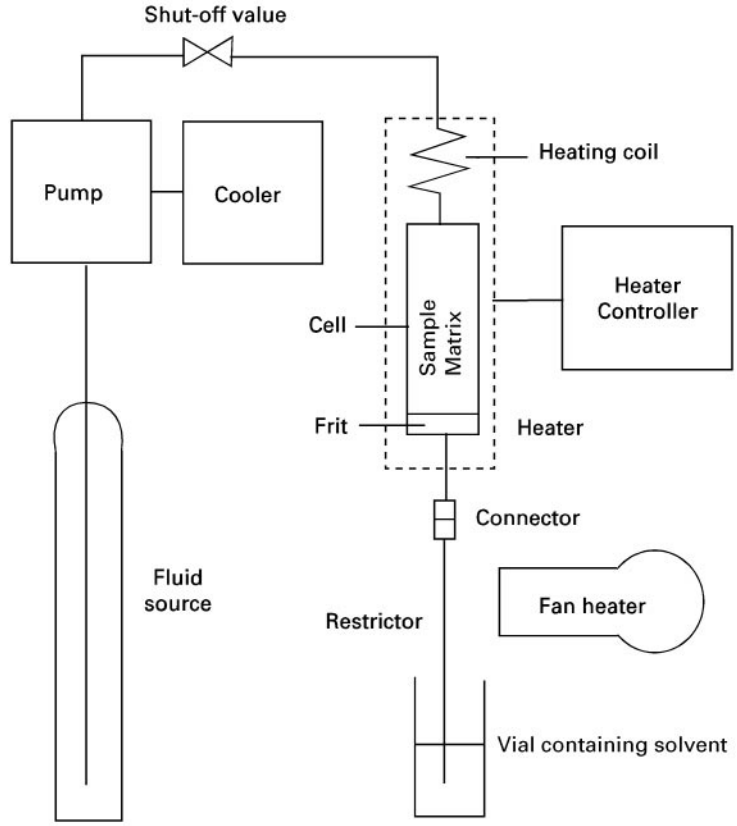

Figure 1 Schematic diagram of a simple system for carrying out SFE on a laboratory scale.

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restrictor and loss of extracted compounds because of inefficient trapping. Furthermore, it does not allow independent control of the flow rate and pressure. More sophisticated commercial methods of pressure control and trapping are available.

In a representative experiment, a 1 mL cell is loaded with 0.5 g of the material to be extracted, (previously dried and ground to particles of 0.1 mm diameter). Carbon dioxide is pumped at a rate of 0.5 mL min^{-1}, measured as liquid at the pump. The temperature is 50°C and the pressure of 400 bar is maintained by a restrictor of 25 μm internal diameter and 12 cm length. The effluent is trapped in 3 mL of dichloromethane, ready for analysis by gas chromatography after an internal standard had been added. The extraction is carried out for 30 min. However, conditions for SFE vary widely and the details for a particular application can be found in the many reports now in the literature.

If a modifier is required, a second liquid pump must be added to the system and the output liquid fed into a mixing chamber just before the shut-off valve in Figure 1. Modifiers are usually added in relatively small amounts, say 5% or 10% by volume. It is possible to purchase cylinders of carbon dioxide already containing small amounts of common modifiers, such as methanol or acetone. If a modifier is used, the trapping solvent is conveniently the same as the modifier, as modifier will precipitate in the collection vial. Trapping is usually more efficient if a modifier is used.

The experiment described above is described as dynamic extraction, as the fluid is continuously flowing through the cell. Static extraction can also be carried out in a similar system if a second shut-off valve is inserted after the extraction cell. During an experiment, the cell is pressurized with fluid and the cell isolated so that contact between the matrix and fluid can occur for a period of about 30 minutes. A short dynamic stage is then carried out to remove the fluid, containing the dissolved extract, from the cell. For a static extraction, a modifier may be added as liquid to the cell before closing it.

SFE can readily be coupled to gas chromatography by passing the restrictor through a septum into the injection port of a chromatograph. This procedure can be much more sensitive, as all the extracted material is transferred to the chromatograph, whereas in an off-line experiment, only a small fraction of the collection solvent will be injected. Thus the procedure

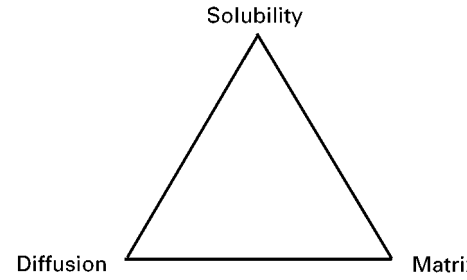
is applicable for example to the analysis of pesticides at low levels. To carry out this procedure, the first section of the chromatographic column is cooled and the carrier gas turned off. SFE is then carried out with the carbon dioxide, or other fluid substance, passing out through the column and the extracted materials depositing at the inlet of the column. SFE is then stopped and the carrier gas passed through the column to flush out the carbon dioxide. The column is then raised to the analysis temperature and chromatography carried out.

Pilot and Process-Scale SFE

The basic process of extraction on a process scale is analogous to that on a laboratory scale and is shown schematically in **Figure 2**. The fluid substance, such as carbon dioxide, is pumped as a liquid and therefore is initially cooled to, say, 5°C, which must allow for some heating during pumping, and kept in a cooled reservoir. A system for adding a proportion of liquid modifier, not shown, may be incorporated. The fluid is then heated to the extraction temperature and pumped into an extraction cell, which is maintained at this temperature. The matrix to be extracted is packed into the extraction cell in a mesh basket to prevent it being carried out of the cell during extraction. Following extraction, the pressure is reduced to precipitate the extract through a control valve. The flow rate of fluid is controlled by the rate of pumping and the pressure in the extraction cell is controlled by the setting of the control valve for a particular pumping rate. Control systems may be used to control the extraction conditions. Reduction of pressure causes cooling of the fluid and so heat input is required, as shown. The precipitated material is collected at the base of the collection vessel, which has temperature control and also pressure control from the control valve on the fluid exit. A series of collection vessels at successively lower pressures may be employed to trap all the extract and separate it into fractions to some extent. A trapping liquid, such as a vegetable oil, may be used on a process scale to give a particular product. Trapping onto a surface, such as active charcoal, may also be used, particularly for volatile products, followed by thermal desorption. On a process scale the fluid leaving the collection vessel is likely to be cooled for recycling.

Mechanisms and Kinetics of SFE

Although extraction is essentially a complex process in which many factors, including procedural parameters, are involved, in a basic theoretical approach the process of extraction can be considered to involve the three factors shown in the SFE triangle below.



The solute must, firstly, be sufficiently soluble in the supercritical fluid to be removed by solution in the fluid flow. If this is not the case, it will be revealed by interpretation of the kinetic recovery curve, as shown below. If solubility is insufficient, the situation may be improved by adding a modifier to the fluid, as described earlier.

Secondly, the solute must be transported sufficiently rapidly, by diffusion or otherwise, from the interior of the matrix in which it is contained. The diffusion process may be normal diffusion of the solute, or it may involve diffusion in the fluid thorough pores in the matrix. The time-scale for diffusion will depend on the diffusion coefficient and the shape and dimensions of the matrix or matrix particles. Of these the shortest dimension is of great importance, as the times depend on the square of its value. Values for this quantity of 1 mm or preferably less are usually necessary.

Thirdly, the solute must be released by the matrix. This last process may involve desorption from a matrix site, passage through a cell wall, or escape from a cage formed by polymer chains. It can be slow and in some cases it appears that part of the substance being extracted is locked into the structure of the matrix. An example is the SFE of additives and lower oligomers from polymers, which can give much lower results than obtained by dissolving the polymer in a solvent, or using liquid extraction at higher temperatures, which swells the polymer to a greater extent. Thus SFE will not always give the total amount of a compound in a sample, only the amount extractable under particular SFE conditions. It may be that the latter is of interest, for example for determination of the migration of additives from polymers into foodstuffs, but if the total amounts are required, SFE may not be applicable. Preliminary experiments, and comparisons with other methods, are necessary. It can be strongly temperature-dependent and thus higher temperatures may improve the situation. The addition of modifiers may often reduce the matrix effect; in fact modifiers are often more important in this respect

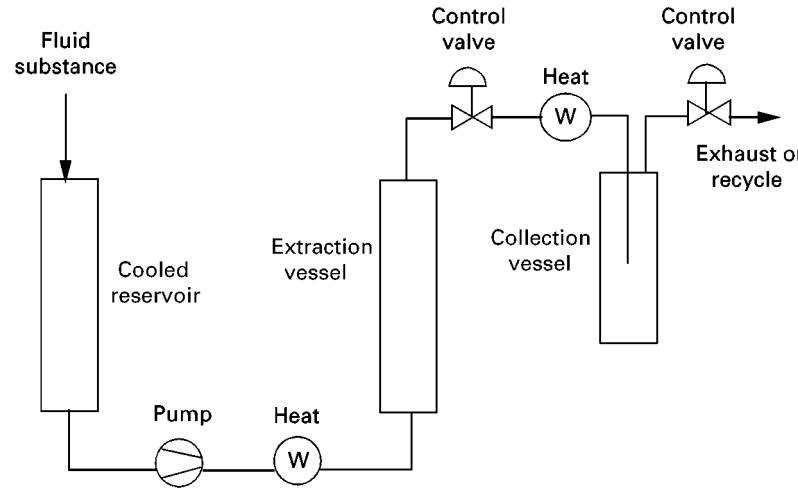


Figure 2 Schematic diagram of SFE on a process scale.

than in enhancing solubility. The mechanism is thought to involve interactions with surfaces. It should be emphasized here that the matrix effect also occurs with liquid solvent extraction. The fact that solvent strength can be varied in a supercritical fluid means that the matrix effect is more obvious in this medium and can be studied in more detail. The advantage is that conditions can often be found in SFE where the matrix effect is minimized.

A related problem is the presence of water. Water is not very soluble in many fluids, such as carbon dioxide, and it can 'mask' the substances to be recovered. The rate of extraction may sometimes be equal to the rate of water removal. It may be necessary to dry the material to be extracted in air or by admixture with a drying agent, such as diatomaceous earth or anhydrous magnesium sulfate. Reduction of the water content of plant material from, say, 80% (as measured by mass loss at 100°C) down to 10% may be desirable, provided valuable volatiles are not lost in the process. However, water may assist extraction by acting as a modifier, as is believed to be the case for coffee decaffeination.

Modifiers or entrainers added to the fluid, as discussed earlier, may be beneficial to any of the above factors. They may improve the solubility of the compounds to be extracted and this was originally thought to be their most important role. However they often improve diffusion by absorption into a polymer and swelling it, for example. Modifiers

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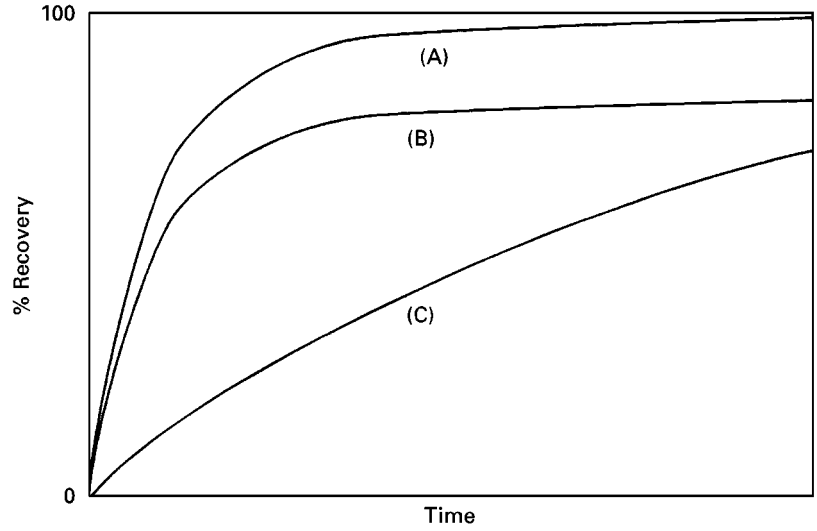

Figure 3 Examples of schematic recovery curves, where recovery is controlled by (A) diffusion; (B) diffusion and matrix effects; and (C) by solubility.

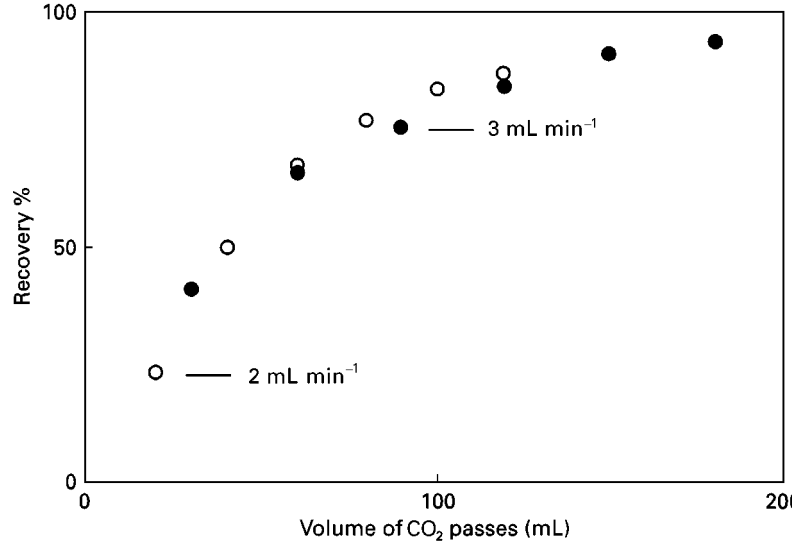

Figure 4 Extraction of lycopene from tomato paste at 100°C and 400 bar at two flow rates plotted against the volume of CO_2 passed.

may improve the matrix factor by adsorbing on surface sites. Modifiers, such as methanol, can reduce the water problem by improving its solubility in the fluid.

Figure 3 shows examples of the types of curves of recovery versus time that can be obtained in SFE. Curve (A) is a typical curve obtained when the process is controlled by diffusion. When matrix effects are significant, the results may have the form of (B). Curve (C) is an example of recovery behaviour when the extracted compound is not very soluble in the extracted fluid. It is thus highly desirable, when developing a procedure for a particular application, to carry out kinetic experiments to obtain curves of recovery versus time. The curves can then be used to investigate the reaction mechanism, as well as determine a suitable extraction time. These developments are detailed in some of the books listed in the bibliography. Kinetic experiments are done by replacing the collection vial periodically. As extraction is faster initially, the time intervals are smaller near the beginning of an extraction. A representative series of times for changing over the collection vial is 2, 5, 10, 20, 30, 40 and 60 min. The total amount extracted can also be compared with liquid extraction.

An example of an experimental recovery curve is now given in **Figure 4** for the extraction of lycopene from 0.5 g of tomato paste, dried by mixing with diatomaceous earth, at 100°C and 400 bar. Flow rates of 2 mL min^{-1} and 3 mL min^{-1} per minute (measured as liquid at the pump) were used, and the results plotted not against time, but against the volume of CO_2 passed (i.e. the time multiplied by the flow rate). The fact that the two curves approximately coincide, indicates that the extraction is principally by the partition of lycopene between CO_2 and the tomato paste matrix, which in turn is related to the solubility of lycopene in CO_2.

Conclusions

Supercritical fluid extraction can be a clean alternative to liquid solvent extraction both for analytical sample preparation and for production scale, because environmentally friendly solvents such as carbon dioxide can be used instead of organic solvents. Some applications are found to be more successful than others. It requires more expensive equipment and a greater commitment to process development than liquid extraction. Nevertheless, it is being applied in specific areas, such as for polymer additives in analytical chemistry and as a method for obtaining valuable compounds from plants on a process scale.

See Colour Plate 44.

See also: **II/Chromatography: Supercritical Fluid:** Large-Scale Supercritical Fluid Chromatography. **III/Environmental Applications:** Supercritical Fluid Extraction. **One-line Sample Preparation: Supercritical Fluid Extraction. Polymers:** Supercritical Fluid Extraction.

Further Reading

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Ultrasound Extractions

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Introduction

Sound is transmitted through a medium by inducing vibrational motion of the molecules forming part of it. Human hearing threshold is reached when the frequency of sound is higher than 16–18 kHz. Ultrasound comprises the region of frequencies between 18 kHz and 100 MHz, the upper limit not being sharply defined (**Figure 1**). This broad region can still be divided into two different regions: power ultrasound between 20 and 100 kHz and diagnostic ultrasound between 1 and 10 MHz. The above classification relies on the capability of energy transmission into the medium at the lower frequencies, which induces the cavitation phenomenon.

Relevant applications of ultrasonic energy include its use in animal communications (e.g. bat navigation and dog whistles), medicine for fetal imaging, underwater range finding (SONAR) and nondestructive testing for metal flaws. Recently, ultrasound has also been considered a potential source for enhancement of chemical reactivity. A large variety of chemical and industrial processes rely on high intensity ultrasonication, e.g., cleaning, drilling, soldering, acceleration of chemical reactions, emulsification, sterilization, flotation, homogenization, dissolution, deaggregation of powder, disruption of biological cells, extraction, crystallization, oxidation, etc. A further advantage of the above-mentioned ultrasound-assisted processes is the relative simplicity of both method development and instrumentation.

A brief description of ultrasound fundamentals as well as a discussion of its applications for solid–liquid extraction is given below.

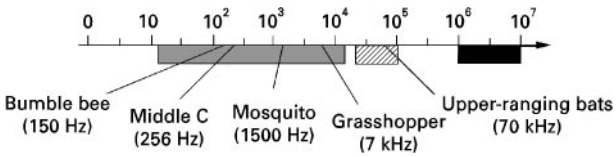

Figure 1 Sound frequencies (Hz, cycles per second). ▩, human hearing 16 Hz–16 kHz; ▨, power 20 kHz–100 kHz (clearing plastic welding sonochemistry); ■, high frequency 1 MHz–10 MHz (medical diagnosis, chemical analysis). (From Mason TJ (1991).)

Fundamental Features of Ultrasound

Vibrations Induced by Ultrasound

Sound waves are usually represented as a series of vertical lines, with intensity being related to separation between them, or as a sine wave where intensity is related to the amplitude (**Figure 2**).

Ultrasonic irradiation of a liquid medium gives rise to an acoustic pressure (P_a) which is added to the hydrostatic pressure (P_h) which exists in the medium. The acoustic pressure depends on time according to the following expression:

$$P_a = P_A \sin 2\pi ft$$

where f is the frequency of the wave (> 16 kHz), t is the time and P_A is the maximum pressure amplitude of the wave. At the point where the lines are close to each other, pressure is higher than normal (i.e. compression region), whereas at the point where the lines are furthest apart, pressure is lower than normal (i.e. rarefaction region).

The intensity of the wave can be defined as the energy transmitted per second per cm^2 of fluid and can be related to P_A as follows:

$$I = P_A^2(2\rho c)^{-1}$$

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